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Use of modified fine needle aspiration for study of glomerular pathology in human kidneys

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Use of modified fine needle aspiration for study of glomerular pathology in human kidneys. In routine fine needle aspiration (FNA) of the kidney, the glomeruli are seldom visualized. They appear as multi-layered, cellular conglomerates and, therefore, are unsuitable for morphological analysis. A novel plasma-clot technique for collection of glomeruli from FNA samples was used in a study of 6 native and 24 transplanted human kidneys with suspected glomerular lesions. This technique produced a satisfactory yield of well preserved glomeruli and enabled the identification of glomerular pathology with the accuracy comparable to that of renal core biopsy. The FNA plasma clot method may prove useful in the study of glomerular pathology under conditions where the use of percutaneous biopsy is conventionally limited or avoided.

Renal tissue biopsy is an invaluable tool for the study of glomerular pathology. However, its use in human subjects is limited in clinical situations in which core biopsy, albeit desirable, does not seem justified because of its potential complications. Such circumstances may occur when the need arises for repeated diagnostic or follow-up biopsies, in states with minimal renal symptomatology such as microerythrocyturia, minimal proteinuria, clinically asymptomatic glomerular involvement in systemic diseases or in the course of overt renal disease in the solitary kidney. Additional material for glomerular study could also be of benefit in renal transplantation where it may help to clarify the mechanisms of post-transplant glomerulonephritis, rejection glomerulopathy and post-transplant amyloidosis. The aim of this study was to evaluate a novel application of minimally invasive, fine needle aspiration technique, modified for collection of well preserved glomeruli. With this technique, we studied 6 native and 24 transplanted human kidneys. To verify the FNA diagnostic accuracy in identifying characteristic glomerular changes, the FNA findings were compared with their parallel renal core biopsies in 11 cases.

Methods

Thirty FNA samples from 6 native and 24 transplanted kidneys were studied. The patients comprised three groups. The first group included six non-transplanted patients aged 3 to 62 years with proteinuria and/or hematuria, and various degrees of impairment of renal function. All patients in this group had renal core biopsies. The second group comprised 14 long-term renal transplant recipients aged 11 to 42 years, with renal graft dysfunction. Similarly, 11 patients in this group underwent renal core biopsies simultaneous with FNA. One additional patient had graft nephrectomy and in two patients no core biopsies were performed. The third group comprised 10 recipients aged 22 to 23 suffering from Familial Mediterranean Fever, all of them having well functioning renal grafts. These patients were included in the study for the purpose of screening for occult glomerular amyloid deposits in their grafts. For ethical reasons, the study protocol in this group allowed renal core biopsies only when previous clinical or FNA evidence of glomerular pathology was obtained. Thus, only one tissue biopsy was done in this group.

The FNA samples were obtained by the classical Helsinki technique [1], using a 25-gauge spinal needle and prepared for glomerular analysis by the S.M. Miller, P. Belitsky and R. Gupta method [2]. After separation of 600 μl alliquot of the aspirate for the conventional cytosmear, the FNA sample suspended in 1640 RPMI solution supplemented with albumin and heparin was centrifuged in gelatin-coated plastic tubes for 10 minutes at 1000 rpm. Following centrifugation, the supernatant was removed and the cellular pellet was entrapped in plasma clot produced by an addition of plasma and thrombin to the sediment. The resulting specimen was wrapped in absorbent paper (absorbent lens cleaning paper, Clay Adams Division of Beckton & Dickenson Co., Parsippany, New York, USA) and fixed in glutaraldehyde for one hour. Following fixation, the specimen was divided into two parts. One for light microscopy was post-fixed in formalin and further processed by routine histological methods. The second part allotted for electron microscopy was post-fixed in osmic acid and embedded in epon. Paraffin sections were stained with hematoxylin eosin, periodic acid Schiff and methenamine silver. Ultrasections were double-stained with uranyl acetate and lead citrate. For diagnosis of glomerular deposits of amyloid, Congo-red stain, light polarizing and electron microscopy were used. Percutaneous renal biopsies were obtained by Travenol Tru-Cut needles and processed routinely.

The FNA specimens and tissue samples were coded and

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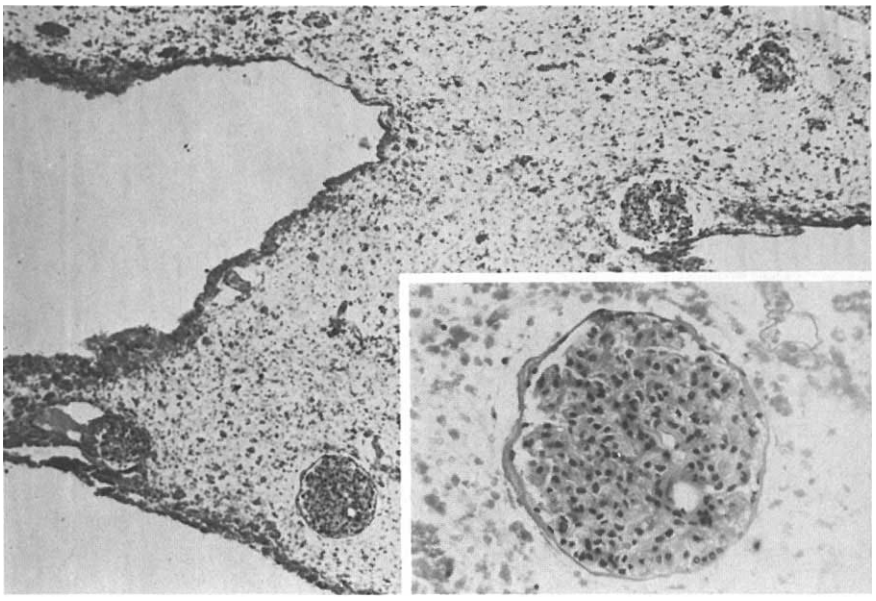

Fig. 1. *Low power magnification of FNA showing glomeruli dispersed in a field of erythrocytes and fibrin (×40).* Inset: High power magnification of a well preserved glomerulus with intact Bowman's capsule (×200).

examined separately by one of us (Prof. M. Ben-Bassat). The reader of pathology was able to distinguish FNA from tissue sample due to characteristic absence of other than glomeruli parenchymal renal elements in sections prepared from plasma clot. However, because of the coding of the specimens at the time of the examination, she had no knowledge of which FNA and tissue samples were taken from the same patient.

Results

Twenty-five of 30 FNA specimens contained glomeruli and occasional tubular fragments surrounded by fibrin and erythrocytes (Fig. 1). The mean total number of glomeruli per FNA sample was 5.6 ± 2.6 (SD) (not inclusive of 5 FNA's without glomeruli) versus 8.2 ± 2.1 (SD) per needle biopsy specimen. In the light microscope, the structural integrity of glomerular tufts was well preserved in most cases (23 out of 25) and showed no distortions or artifacts. The Bowman's capsule was preserved in about 30% of the specimens (Fig. 1). The ultrastructural glomerular details examined in eight FNA samples were generally slightly less apparent in FNA than those in tissue biopsies but were found to be adequate for pathological analysis in all cases studied. The quality of hematoxylin eosin, periodic acid Schiff, methynamine silver and Congo-red staining paralleled that of tissue samples.

The immunoperoxidase staining, which was performed in one case (No. 8) showed a granular pattern of reaction of IgG, IgM and C3 antisera with glomerular basement membrane.

The analysis of glomerular findings carried out in 25 aspirates revealed normal glomerular morphology in 10 specimens, pathological changes in 13 and collapsed glomeruli not suitable for morphological examination in 2 aspirates. The glomerular alterations covered a wide range of glomerulopathies (8 cases, Fig. 2), amyloid deposition (2 cases, Fig. 3), necrotic glomeruli (1 case) and mesangial changes (2 cases, Table 1).

In all cases with glomerular lesions, except two in which core biopsy was not performed, the FNA diagnostic interpretation was found to be concordant with that of the conventional histology from renal biopsies (Table 1). The FNA procedure was performed without anesthesia in 23 patients, local anesthesia was used in FNA biopsies of five native kidneys, one child was aspirated under mild sedation and another under general anesthesia. In native kidneys, the fine needle aspirations were performed under ultrasonographic (5 cases) and fluoroscopic (1 case) guidance. In contrast, all transplanted kidneys were located only by palpation. There were no complications associated with FNA and the procedure was well tolerated by all patients. Following Tru-Cut biopsies, two episodes of transient hematuria were observed. The FNA sample preparation time before commencement of routine histological processing ranged, in this study, from 13 to 17 minutes. Diagnostically usable material was obtained in 23 out of 30 patients (77%).

Discussion

Potential complications of percutaneous renal biopsy preclude its use in many clinical settings of great interest to both clinicians and scientists. One of these is minimal proteinuria and microhematuria which may for a long period of time precede an overt renal disease. The traumatic nature of percutaneous biopsy also very frequently becomes an argument for restraint in application of serial renal biopsies in following-up the course and response to treatment of various glomerular lesions. Among these is post-transplant glomerulonephritis which has emerged as one of the major causes of long-term post-transplant morbidity, becoming second only to chronic rejection. Its pathogenetic mechanisms and even its true frequency are still unknown, due to the insufficiency of histologic material. Moreover, the additional information on the state of renal glomeruli can be helpful in investigation of renal graft dysfunction during viral infections, chronic rejection and in systemic diseases of the recipient, such as diabetes, lupus and amyloidosis. Therefore, it is evident that a safe and simple technique for glomerular collection is required to increase the yield of clinically useful information on glomerular morphology in many conditions in which conventional renal biopsies are

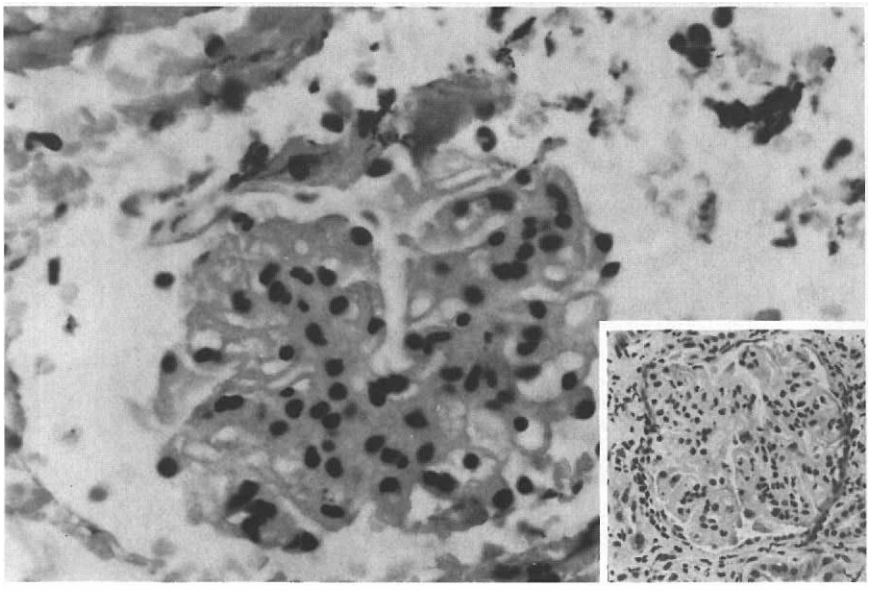

Fig. 2. *FNA from patient No. 8 showing a glomerulus with morphological features of membranoproliferative glomerulonephritis (×200).* Inset: Glomerulus from needle biopsy in the same patient, to be compared with FNA (×100).

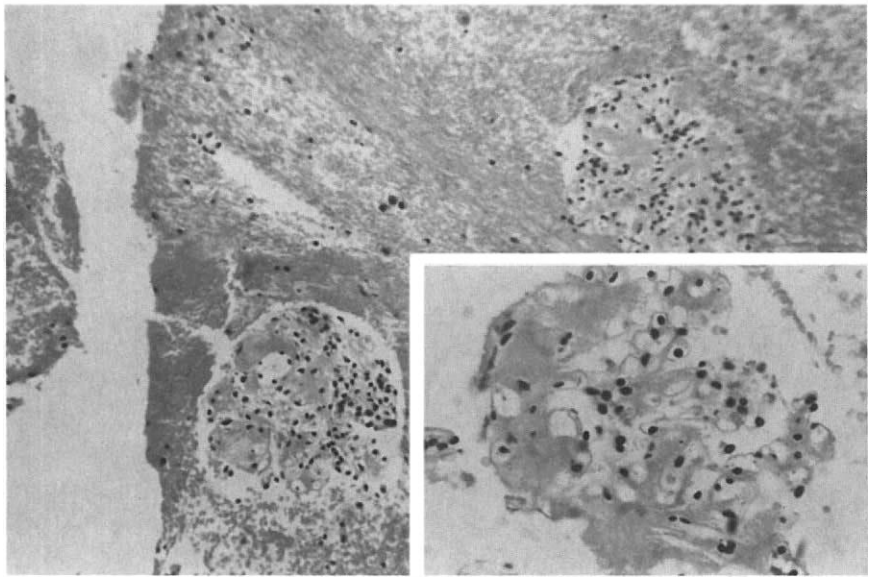

Fig. 3. *FNA containing two glomeruli which showed positive reaction for amyloid (×100).* Inset: High power magnification of a glomerulus with amyloid deposition (×200).

withheld for fear of complications. The search for the minimally traumatic procedure resulted in a study of FNA as an alternative to the core biopsy method for glomerular sampling. In 1978, Pasternack et al published a first comprehensive report of the FNA method for glomerular collection utilizing a modified cone-shaped fine needle and a specially constructed sieving device [3]. This and other later methods, while enabling preparation of histological sections from fine needle aspirates, require special equipment and processing techniques [3, 4].

In 1987 the Canadian group of Miller, Belitsky and Gupta presented a simple method for histological vizualisation of glomeruli in FNA samples treated with the plasma-clot technique [2]. With the use of this technique, which in contrast to other FNA methods of glomerular sampling can be performed without any special collecting and processing devices, the same group achieved a high yield of well-preserved glomeruli in a dog renal transplant model, and on one occasion diagnosed post-transplant glomerulonephritis in a human subject [5].

The authors showed that in the study of glomeruli obtained by FNA, all routine histochemical stains worked effectively. They also emphasized that in the case of post-transplant glomerulonephritis, the morphological details of the glomeruli obtained from FNA were comparable with those from core biopsy.

In our study we evaluated the technical aspects and diagnostic accuracy of the FNA plasma-clot technique in the study of glomerular pathology in a group of human subjects. The usefulness of this method was evaluated in pediatric and adult

Table 1. Glomerular pathology in FNA from 8 transplanted and 5 native kidneys

Pat. no.	Age/ sex	Clinical features	Kidney source	FNA		Tissue biopsy	
				Light microscopy	Electron microscopy	Light microscopy	Electron microscopy
1	22/F	Stable function, mild proteinuria	Cadaveric transplant	Amyloid deposition	Amyloid fibrils	Amyloidosis of kidney	Amyloid fibrils
2	42/M	Nephrotic syndrome, hypertension, deteriorated function	Living related transplant	2 Normal glomeruli	EDD in an obliterated segment	FSGS in 20% of the glomeruli	EDD in obliterated glomerular segments
3	21/M	Mild proteinuria, deteriorated function	Living related transplant	Susp. thickening of GBM	MGN—Stage 1	Not done	Not done
4	32/M	Diabetes mellitus, proteinuria, deteriorated function	Cadaveric transplant	Diabetic nephropathy	Thickening of GBM and mesangium	Diabetic nephropathy diffuse nodular pattern	Thickening of GBM and mesangium
5	33/F	Transplant failure, proteinuria, hematuria	Cadaveric transplant	Necrotic glomeruli	Not done	Ischemic infarction of kidney	Not done
6	11/F	Proteinuria, Micr. hematuria, stable function	Cadaveric transplant	MGN? RG?	MGN—Stage 2	Not done	Not done
7	29/F	Deteriorated function	Cadaveric transplant	MGN? RG?	Thickening of lamina rara interna—RG. no EDD	MGN? RG?	Thickening of lamina rara interna—RG
8	25/M	Nephrotic syndrome	Cadaveric transplant	MPGN	MPGN—Type 3 subepithelial pattern	MPGN	MPGN—Type 3 subepithelial pattern
9	3/F	Steroid-dependent nephrotic syndrome	Native kidney	Mesangial thickening	Not done—no glomeruli	15 Normal glomeruli	Effacement of foot processes
10	26/M	Proteinuria, arthralgia, ANF positive, susp. SLE	Native kidney	DPGN	Not done	DPGN	EDD in all aspects of glomerular tuft
11	43/M	Nephrotic syndrome	Native kidney	MPGN	Not done	MPGN	Subendothelial EDD MPGN—type 1
12	62/M	Steroid-resistant nephrotic syndrome	Native kidney	Slight mesangial cell proliferation	Effacement of the foot processes; no EDD MCD	Slight mesangial cell proliferation	Effacement of the foot processes; MCD
13	18/M	Nephrotic syndrome, stable function, susp. F.M.F.	Native kidney	Amyloid deposition	Not done	Amyloidosis of kidney	Not done

Abbreviations are: EDD, electron dense deposit; FSGS, focal segmental glomerulosclerosis; MGN, membranous glomerulonephritis; MCD, minimal change disease; RG, rejection glomerulopathy; MPGN, membranoproliferative glomerulonephritis; LN, lupus nephropathy; GBM, glomerular basement membrane; DPGN, diffuse proliferative glomerulonephritis; FMF, Familial Mediterranean fever.

patients in a variety of clinical conditions, such as native kidneys with clinical signs of renal disease, failing renal transplants, and stable renal transplants suspected of occult amyloidosis. The analysis of aspirated specimens showed that a sufficient amount of well-preserved glomeruli, suitable for all routine histochemical and immunological stains and electron microscopy could be obtained in nearly 80% of the samples. The 20% failure rate to obtain glomeruli in this series reflects our initial inexperience with the technique of plasma clot preparation and not with the renal aspiration itself. In our opinion, the sampling failures are still more likely to occur in native kidneys because of their concealed location. These, however, could be amended by the repetitive aspirations which are generally very well tolerated by the patients. Although in ultrasections from FNA, the glomerular details were somewhat less apparent than in core biopsies, the characteristic glomerular features of renal disease could be recognized in FNA with the same diagnostic accuracy as in core biopsy (Table 1).

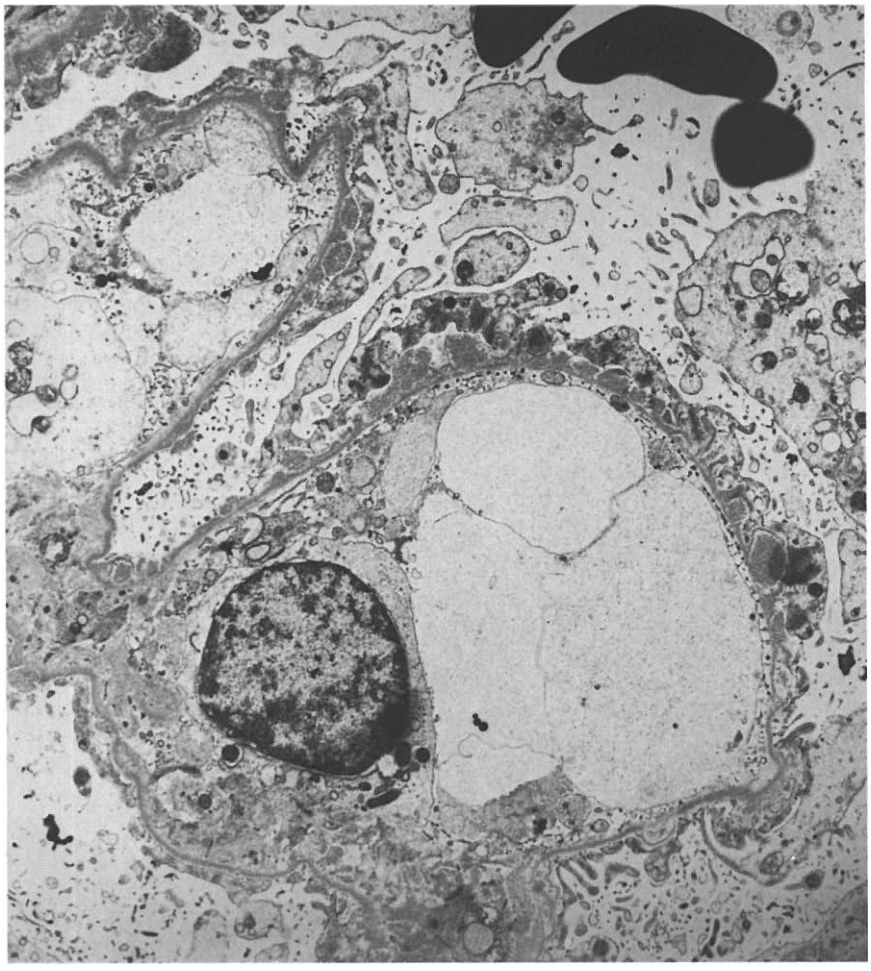

Fig. 4. *Electron micrograph of FNA from patient No. 6 exhibiting stage II of membranous glomerulonephritis (×8,250).*

The pathological findings in needle biopsies and FNA are summarized in Table 1. Some of these are discussed here in more detail. In case No. 2, the diagnosis of focal segmental glomerulosclerosis (FSGS) was made by electron microscopy (Fig. 1). The paraffin sections of FNA showed only two glomeruli which appeared normal. The examination of the material from the same FNA sample embedded for electron microscopy revealed an involved glomerulus showing segmental obliteration of glomerular tuft. The ultrathin section from this glomerulus showed electron-dense deposit in the involved segment. The diagnosis of FSGS in this case was confirmed by core biopsy showing focal segmental involvement in 20% of the glomeruli. In cases Nos. 6 (Fig. 4) and 7 (Fig. 5), the electron microscopic findings in FNA specimens also resolved the problem of differential diagnosis between membranous glomerulonephritis (MGN) and rejection glomerulopathy (RG), demonstrating the typical behavior of MGN electron-dense deposits in case No. 6 and the characteristic RG thickening of the lamina rara interna in case No. 7.

In another case (No. 5), the tissue biopsy was not done simultaneously with FNA. The examination of FNA obtained from a renal transplant which suddenly ceased to function, showed necrotic glomeruli and led to a presumptive diagnosis of graft infarction. An immediate exploration was terminated in graft nephrectomy. The removed kidney showed an extensive necrosis due to recent thrombosis of renal artery. In case No. 8 diagnosed as membranoproliferative glomerulonephritis (MPGN), an immunoperoxidase study of FNA sample was performed. In this case the reaction of IgG, IgM and C3 antisera with the glomerular capillary basement membrane was detected by a distinctly positive immunoperoxidase staining. Another type of glomerular lesion was found in FNA taken from a renal transplant recipient suffering from Familial Mediterranean Fever and receiving colchicine prophylaxis as well as prednisone-azathioprine. Despite the graft stable function and only minimal proteinuria, well below the nephrotic range, massive deposits of amyloid were found in the majority of the glomeruli. This finding was confirmed by percutaneous biopsy.

We conclude that in this study fine needle biopsy was proven to be of value in providing reliable diagnostic information on various patterns of diffuse glomerular diseases. This technique, capable of producing only isolated glomeruli, obviously is not suitable for study of renal vascular, tubular and interstitial inflammatory and metabolic disorders which should be continually evaluated by conventional tissue and aspiration biopsies. The FNA plasma clot technique, having the advantage of being

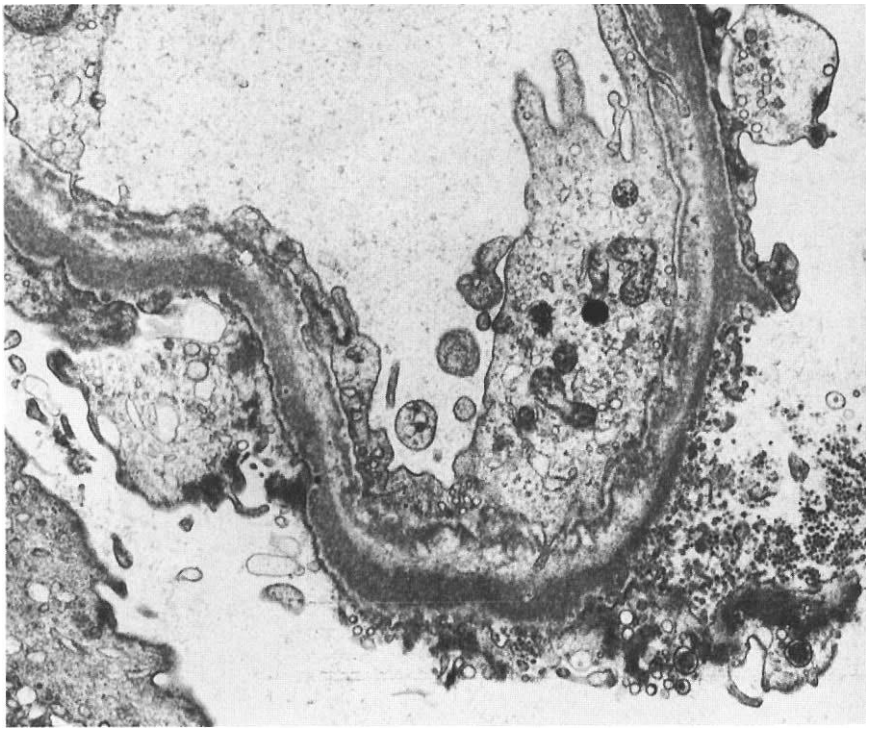

Fig. 5. *Electron micrograph of FNA from patient No. 7 exhibiting a peripheral capillary loop with thickened lamina rara interna, compatible with rejection glomerulopathy.* EDD were not identified (×17,000).

a minimally invasive, rapid, simple-to-perform and easily repeated procedure, could be employed as complementary to the tissue biopsy method for diagnosis and, especially, for monitoring the kinetics of a wide variety of diffuse glomerular lesions in native and transplanted kidneys.

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